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A search for missing pieces of the puzzle; the development of asthma and atopy

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

2009

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Reijmerink, N. E. (2009). *A search for missing pieces of the puzzle; the development of asthma and atopy: innate immunity genes and environment*. [Thesis fully internal (DIV), University of Groningen]. [s.n.].

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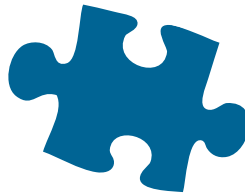
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Chapter 1

Gene-environment interaction and respiratory disease in children

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Eur Respir Mon 2006;37:108-119



Introduction

It remains a big puzzle in complex lung diseases, like asthma, to determine the exact genetic aetiology of the disease. The main reasons for this are that the disease is polygenic, the interaction between genetic (host) and environmental factors is involved, and that there is a wide heterogeneity of asthma phenotypes.¹ A genetic basis for asthma has been demonstrated in numerous family studies. The findings were consistent, irrespective whether the study was performed in twins, in trios, or by segregation analysis of extended pedigrees.² Many investigators have found evidence of linkage between genetic markers and asthma as well as its associated phenotypes, and until now seven genes have been found by positional cloning.¹

The role of environmental exposures to viruses, non-specific stimuli, and allergens in the daily morbidity of asthma and atopy has been recognised for decades. An example of the direct relationship between exposure and morbidity is the early and late asthmatic reaction that occurs after exposure to house dust (mite), and the subsequent increase in response to non-specific stimuli.³ Thus nowadays it is accepted that next to the genetic basis, the environment plays an important role in asthma development also.

More specific genes, environment, as well as their interactions have been recognised as important and crucial factors in the development of asthma and atopy.⁴⁻⁷

Notwithstanding the progress that has been made over the past decades, it has still not been fully elucidated yet which major and minor genes are responsible for the development of asthma and atopy nor how the interaction is between the already found genes and to what extent the expression of these genes is dependent of other environmental and endogenous factors. Additionally the mechanisms of gene-environment interaction has been subject to different interpretations, as recently discussed.⁶⁻⁹

Gene-gene interaction

Gene-gene interaction in the development of lung disease has extensively been investigated in cystic fibrosis.¹⁰ Already soon after the discovery of the ΔF_{508} mutation in the CFTR-gene, on chromosome 7q, it became clear that there is a great variability of pulmonary phenotypes and survival in cystic fibrosis, even among patients homozygous for the most prevalent mutation ΔF_{508} .¹¹ This variability could partly be explained by modifying environmental factors, like severe pulmonary infections, nutritional status, early development of liver disease and other concomitant diseases.¹² Recently it has been shown that additional genetic variation (i.e. presence of “modifier” genes¹³) contributes to the expression of the final phenotype as well. This has been tested in a few of the ten genes on chromosome 19q13.2.¹¹ In a large North American cystic fibrosis population it has been shown that polymorphisms in the promoter and in the codon 10 region of transforming growth factor β_1 (TGF- β_1) gene on chromosome 19q13.2

is associated with pulmonary phenotypes predictive of the long-term outcome of patients with cystic fibrosis homozygote for ΔF_{508} in the CFTR-gene. Interestingly, recent association studies have also linked these TGF- β_1 polymorphisms to atopy, asthma and chronic obstructive pulmonary disease (COPD).¹⁴⁻¹⁸ These polymorphisms of TGF- β_1 are functional in that they are related to abnormalities of the airways like induction of extracellular matrix in asthmatic airway smooth muscle and orchestration of airway remodelling.^{19,20}

Another example of gene-gene interaction was reported by Blumenthal et al in the collaborative study on the genetics of asthma (CSGA-study) performing a nonparametric gene analysis approach. When conditioning on chromosome 11q, there was increased evidence for linkage in four other chromosomal regions: 5q, 8p, 12p and 14q but not for 20p.²¹ Gene-gene interaction analysis has also been performed with candidate genes of asthma. IL-13 and IL-4RA are both key molecules in Th-2 signalling.²² Variations in the IL-13 gene have been associated with bronchial hyperreactivity (BHR), asthma susceptibility, and IgE. While a borderline significant association was observed between polymorphisms in IL-4RA and BHR and asthma, both BHR and IgE are risk factors for asthma and interaction between the genes could be expected. Indeed when both genes in combination were analyzed, individuals with the risk genotypes had a nearly 2.5 times greater risk of developing asthma than individuals with either genotype alone and five fold compared to those without these genotypes.

These findings make it likely that gene-gene interaction plays an important role in asthma like in cystic fibrosis, although the mechanisms by which interaction between 'modifier' genes and the candidate asthma genes act are still to be unravelled. It also stresses the importance of studying gene-gene interaction in complex diseases, since this may elucidate pathways that play a role in disease development, disease severity and disease progression.

Gene-environment interaction

The environment was pointed out as one of the factors playing a role in the pathogenesis of asthma and atopy. Strong indications were exacerbations of allergic rhinitis during particularly the pollen season and the beneficial effect of house dust mite avoidance in the mountains on asthma severity, bronchial hyperreactivity and medication use.²³ However, the allergic reactions occur in patients with established disease, and this does not prove whether the environment also contributes to disease development, disease severity, and/or disease progression. Epidemiologic studies have shown that exposure to allergens is related to the development of allergic diseases.^{24,25} Furthermore, in farmer studies and areas with high infection rates the environment also can have a protective effect on the development of asthma and allergy.^{26,27} Since, as previously mentioned, the role of genes in asthma are also established, it is plausible that genes constitute the link between the environment and development of atopy and asthma. Gene-environment interactions can be assessed in case-control and cohort studies as well as in family-based genetic studies. Twin studies have provided suggestive evi-

dence for both genetic and environmental contributions to asthma. The heritability of asthma has been reported to vary between 60 and 80 %, leaving a remaining 20 to 40 % for environmental contributions.²⁸⁻³⁰ Until recently, the genetic and environmental susceptibility were studied separately, or the potential interaction between these two sources was evaluated by stratifying the effect of exposures by family history.³⁰ Significant interactions, demonstrated in both linkage and association studies, indicate that many early life exposures influence the risk for asthma and its related phenotypes in a genotype-specific manner. These early life exposures include exposure to endotoxins³¹⁻³⁴, viruses^{5,35}, pets^{36,37}, day-care environment⁵, and environmental tobacco smoke (ETS).³⁹⁻⁴²

Several models of gene-environment interactions in asthma and atopy have been suggested by Vercelli, Martinez and modified by Ober.^{43, 6,7} For example the CD14 gene is considered as a potentially critical player in the gene-environment interactions leading to asthma and atopy. Several studies have shown association with variations in the CD14 gene and atopic phenotypes, like IgE.^{9,43,44} The importance of the gene is also confirmed by genetic linkage studies that suggest that one or more loci on chromosome 5q control for the height of serum IgE levels.⁴⁴⁻⁴⁶ Considering gene-environment interactions theoretically several models can be postulated as already published by Martinez and Ober.^{6,7}

The first model is that the phenotype comes to expression when the genotype is present, and the environmental factor does not have an influence on the onset of disease (figure 1).

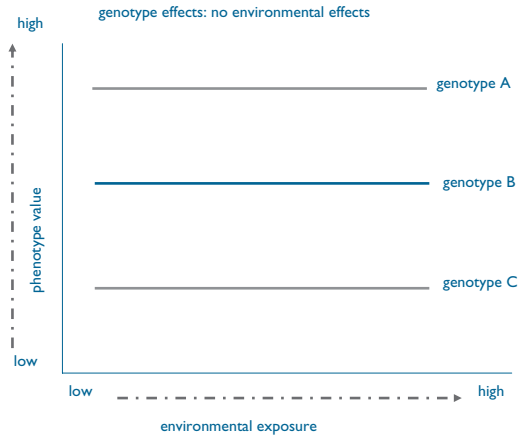


Figure 1.
The association between a phenotype (y-axis) and environmental exposure (x-axis) is plotted for different genotypes. There is no environmental interaction for any genotype.

Thus, the strength of the expression of the phenotypes depends on the genotype and is not influenced by environmental exposure. In the real life situation it is not easy to find a good example. An already mentioned example is cystic fibrosis where the severity of the disease is predominantly determined by the genetic effect and the gene-gene interaction as already discussed before.¹⁰ Nevertheless, also in cystic

fibrosis infections as exogenous factor and pancreatic insufficiency as endogenous factor contribute to the expression of the severity and prognosis of disease.

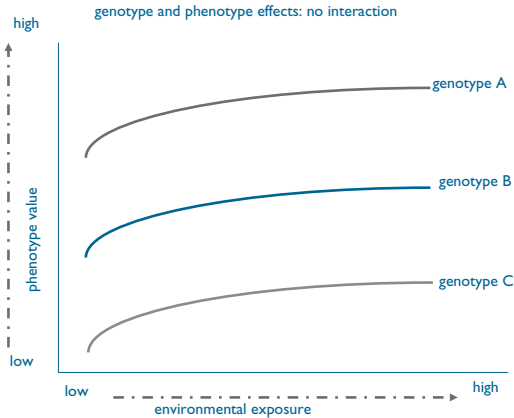


Figure 2.
There is no gene-environmental interaction, therefore the curves for the three genotypes are parallel

In the second model (figure 2), the environmental influence is the same for all genotypes, regardless of the level of exposure, and the influence of the genotypes on the strength of the phenotype is always identical. The consequence of this is that in all environments the estimated heritability of a disease is always similar. If the studies are well designed and well performed the results of the genetic studies will be highly reproducible irrespective of the populations, with the exception that not, like in cystic fibrosis, gene-gene interactions are crucial in the expression of the phenotype. A well known example is phenylketonuria (PKU) a recessive disorder of metabolism in which phenylalanine cannot be converted to tyrosine. The gene for phenylalanine hydrolase has been cloned and mapped to chromosome 12q24.1.⁴⁷ More than 240 mutations have been defined in this disease. There is only little variation in the presentation of the disease between the different genotypes in the presence of phenylalanine in the diet. Restriction of dietary intake of phenylalanine can in all patients with PKU completely prevent disease development in all genotypes.

The third model (Figure 3) presents that the extent of influence of the genotypes on the phenotypes can vary in different environments. Clear examples are the high exposure to microbes in the farming environment and the low exposure to house dust mite in high mountain environment. Both environments decrease the expression of the asthma phenotype, the former by high exposure to microbes and the latter by strong reduction of house dust mite exposure. Immunologically this can be explained by maternal exposure to environments rich of microbes, these exposures eventually determine the priming of the unborn child's immune response. For example, research has shown that these prenatal exposures affect the expression of the Toll like receptors 2 and 4 and CD14 in school age children⁴⁸ These receptors are important in the development and maintenance of the immune system.

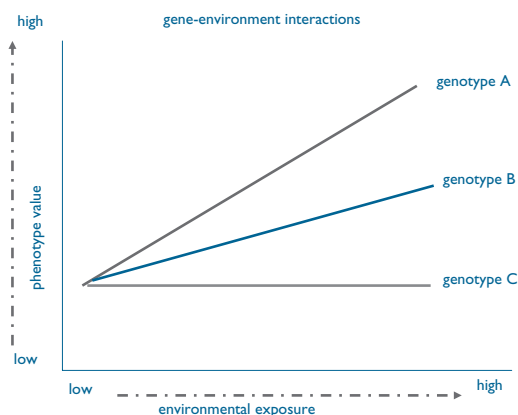


Figure 3. Model of reaction of a case in which gene-environment interaction is present. The variation is explained by gene-environment interaction.

The last model of interaction is presented in figure 4.

The effects of the genotypes are different and cannot be predicted at the different levels of exposure. It is more likely that those with genotype C have the phenotype studied when exposed at low level, whereas those with genotype A are protected. Conversely, at high exposure individuals with genotype A are at increased risk to express the phenotype and individuals with genotype C are protected.

In the review of Ober and Thompson ⁷ a list is published of the known asthma and atopy genes found by positional cloning following linkage studies and candidate gene studies. Despite the large number of successful studies there is not a single gene that has been replicated in all studies and the information of the interaction between the environment and these genes is only limited and not always consistent.

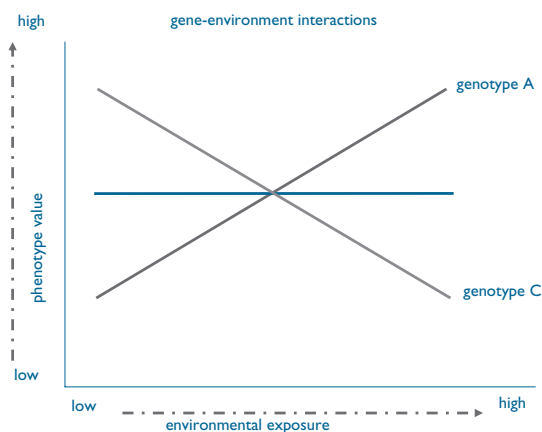


Figure 4. The allele is associated with increased expression of the phenotype which will depend on the degree of exposure. At low levels of exposure, expression of the phenotype is higher for genotype C; at lower levels of exposure, expression of the phenotype is higher for genotype A

An important example of gene-environment interaction is the CD14 genotype, endotoxin exposure and asthma i.e. the functional promoter polymorphism, -159C/T, in the gene encoding the monocyte receptor for endotoxin i.e. CD14. Children with the TT genotype had reduced serum levels of circulating soluble CD14 levels and IgE.⁴⁹ The association with the T allele and reduced risk for atopy was replicated in some, but not all, subsequent studies.⁵⁰ However, studies in a farming population revealed an association between the T allele and increased risk for atopy.⁵¹ It leads to the suggestion that the CD14 variant, CD14-159C/T interacts with environmental levels of endotoxin to determine whether an individual is at risk or even protected from asthma and atopy.⁵² This has recently been confirmed in Barbabos children, in whom the TT genotype was protective against asthma in environments with low house dust endotoxin levels, but associated with risk for asthma in children from homes with high levels.⁵³ More studies are underway especially in farmer children and children living in a Steiner lifestyle environment.

Figures 1–4 give an impression of possible relationships between genes and environment. Asthma is a complex disease in which many different genes and multiple environmental factors (endotoxins, air pollution, viral infections, bacterial infections, food, pesticides, heavy metals, environmental tobacco smoke exposure, etc.) play a role. Thus, given the myriad possible interactions between these genes and environmental factors still is a simplification of one reality. Many feel that investigating these

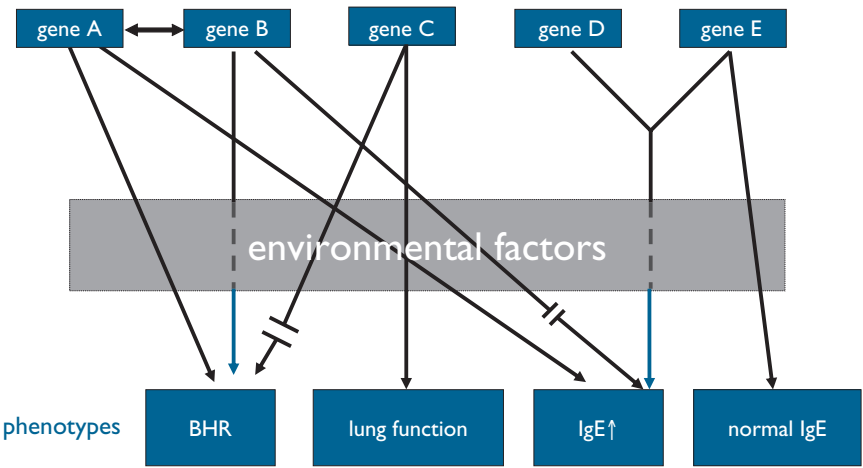



Figure 5. The possible role of genes in the expression of phenotypes and the interaction with the environment. Gene A has a direct effect on BHR without any effect of the environment, while the environment affects the influence of gene B on BHR is leading to increased BHR. Gene C has no effect on BHR and is also not influenced by the environment but is directly related to lung function. Gene D and E only initiate an elevated IgE when both genotypes are present and are influenced by the environment.



relationships is like searching for needles in a haystack or looking at a mathematical model with an infinity number of variables.

The best defined phenotypes for asthma and atopy are bronchial hyperresponsiveness, lung function and IgE. In figure 5 is shown the possible role of genes in the expression of phenotypes and the interaction with the environment. Gene A has a direct effect on BHR without any influence of the environment, while the environment affects the influence of gene B on BHR leading to increased BHR. And gene C has no effect on BHR and is also not influenced by the environment but is directly related to lung function. Gene D and E only initiate an elevated IgE when both genotypes are present and are influenced by the environment.

In genetically manipulated plants and in invertebrates where the genetic traits and the environments can be fully controlled a wide variety exists in phenotypes. This effect is ascribed to phenotypic plasticity which is the development of different phenotypes for the same genotype in the same environments.⁵⁴ Studies in human beings on the relationships of asthma, allergies and environmental factors investigate an immense number of variables which makes these studies very complex and generally offer multi interpretable results. Phenotypic plasticity likely adds to this complexity in men as well.

In utero environment

It has been assumed for a long time that the safest place for the child is the womb, since it protects the genes from any environmental influence. However, it becomes more and more clear that many intrauterine factors can play a role in the development of the respiratory system and the evolution of the immune system. The target of a toxic insult to the lungs during its development likely involves the disruption and/or alteration of a specific molecular signal or transcription factor but, to date, little information is available as to the precise effect of such exposures. An important aspect is timing of exposure during development which appears to be critical to its effects. For example, maternal malnutrition during gestation may significantly retard fetal growth and the development of the lungs, leading to compromised lung function throughout life.⁵⁵ In contrast, exposure to environmental toxicants such as second-hand-cigarette smoke may actually accelerate the maturation of specific cell types in the fetal lung.^{56,57} The results of such an effect on overall lung function changes from the newborn to the adult age are unknown. In general, very little is known regarding the precise effects of maternal personal exposure, like vitamin intake, smoking and nutritional factors, air pollution, viral infections, etc. on the fetus. It is likely that the exposure affects growth changes of the respiratory system which effects may continue after birth. A limitation in the research of prenatal effects on the development of the respiratory system is that for example exact lung function measurements only can be reliably and on a large scale performed from about 2-6 years of age, dependent on the method of lung function measurement used. Consequently, prenatal and postnatal effects are difficult to disentangle. Nevertheless, it has been suggested that changes of


the respiratory system later in life are already measurable shortly after birth.⁵⁸ Children from mothers with asthma have a greater risk of asthma compared to children of fathers with asthma, and refer to the importance of the prenatal environment on subsequent risks. This 'parent-of origin' effect, in which an allele is associated with asthma or atopy only when it is inherited from the mother, has been confirmed in several studies.^{59,60} A study in 200 Dutch families showed that the influence of susceptibility genes for asthma might become apparent only with exposure in utero and early childhood to cigarette smoke.⁶¹

The above studies provide strong circumstantial, though not physiological, evidence that in utero and early childhood exposures may contribute to disease development early or later in life in interaction with genetic factors.

Sex as an endogenous factor

The influences of maternal and paternal history of atopy and asthma on asthma in the offspring differ, as already stated. In addition, studies on cord-blood IgE show that the influence of maternal history of atopy or asthma is stronger in boys than in girls, suggesting that hormonal factors in the offspring may modify the effects of maternal or paternal inheritance.⁶² During childhood and adolescence, boys are nearly twice as likely as girls to develop asthma and this continues until the age of about 14 years.^{63,64} A change to female predominance occurs during late adolescence in females, which exists throughout adulthood, and asthma tends to be more severe in female adults.⁶⁵⁻⁶⁷ The exact mechanisms of these differences on the molecular and genetic levels are unravelled. Before puberty no differences are observed in the production of sex hormones, which changes during puberty leading to the typical differences between males and females. Already from the onset the airways of females are smaller than from males. From this perspective it might be expected that girls have more respiratory symptoms than boys. This is however in contrast with what has been found in boys and girls. Therefore it is evident that the differences in respiratory symptoms, allergy and asthma between boys and girls can not be accredited to anatomical differences in the respiratory system. An explanation for this shift might be the presence of important genes on the x-chromosome which are switched off during childhood and switched on during puberty and adulthood. During the switch off phase in childhood the influence of the genes is minimal and makes that the immune system behaves similar in boys and girls. During puberty and adulthood the genes on the extra x-chromosome are switched on which may induce a predominant influence on the disease expression and severity of asthma. Whether these genes on the X-chromosome are directly responsible for this gender change or whether they act as modifier genes in combination with switched on mechanisms of the hormone chromosomes is not clear.

Given the two alleles of X-chromosome in girls and, one in boys, it is attractive to investigate whether specifically SNP's in genes that are located on the X-chromosome are associated with asthma development and/or severity in girls or females. Candidate genes on the X-chromosome with a possible linkage to asthma and atopy are the Toll-like receptor 7 (TLR-7) and Toll-like receptor 8 (TLR-8) both located at Xp22.3-Xp22.2 play-



ing a role in both innate and adaptive responses. TLR-7 receptors are mainly expressed in the lung and placenta, whereas the TLR-8 receptors are mainly expressed in the lung and peripheral leukocytes.⁶⁸ Viral products may activate TLR-7 or may generate a ligand that interacts with TLR-7, besides T-regulatory cells express TLR-7, and 8.⁶⁹ Another gene is the IL-13 receptor α_1 (IL-13 α_1) and IL-13 receptor α_2 located at Xpter-Xqter and Xq13.1-Xq28 respectively. The receptor for IL-13 is composed of the IL-13 α_1 and one of the forms of the IL-4 receptor on chromosome 5q.^{70, 71} IL-13 is secreted from CD4(+) T cells, mast cells, basophils and eosinophils, is a central mediator of allergen-induced airway hyperresponsiveness and is associated with elevated serum IgE levels.^{72,73} A noncoding variant of IL13R- α_1 is associated with high IgE levels especially in males suggesting an X-linked inheritance of high IgE levels.⁷⁴⁻⁷⁶ Another important gene located on chromosome Xq13.2-21.1 is Cysteinyl-leukotriene receptor 1 (cysLT₁) playing via LTC₄, LTD₄, and LTE₄ a role in mediating human asthma and activate at least the 2 receptors cysLT₁ and cysLT₂. Activation of these receptors induces many of the biological effects relevant in the pathophysiology of asthma.^{77,78} These genes on the x-chromosome have a direct relationship with asthma and allergy. Which exactly are the functions of these genes and how the interplay with asthma genes is (gene-gene interaction) and whether also other genes on the X-chromosome play a role in the gender related differences in disease expression has to be elucidated.

Conclusions and future perspectives

It is evident that the environment plays a pivotal role in the development and in the severity of asthma and allergy. The exact role of the environment in disease development and progression still has to be unravelled. This is relevant since it offers opportunities for early and life-long intervention. Knowledge about the genetics of these diseases and the interplay with the environment is furthermore essential. However, since the genetics of asthma and allergy as polygenetic diseases is extremely complex, the discovered genes only partly shed light on the risks of development and progression of diseases. Thus research in gene-environment interaction may still be a matter of trying to find escapes in this labyrinth. Genome wide screens are very expensive and the signals, although interesting, so far, do not provide us with the final solution to solve the puzzle why some individuals develop allergy and asthma and others don't. More costly and intensive ways to approach this issue are fine-mapping strategies of chromosomal regions genome-wide association mapping. These need large cohorts given the multiple genetic and environmental factors involved. Recent collaborative studies of different genetic centres involved in large (birth)-cohorts like in GABRIEL increases the power extensively and the expectations are that this will provide us with new and important information. Another approach is to perform comparative studies translating findings in animals, for example mice, to men. Furthermore, the introduction of the micro-arrays or DNA-chip technology offers the opportunity to high-throughput analysis of biological systems and to investigate a high number of

genes at one time, thus allowing genetical-genomics approach towards identification of genes in asthma and its related phenotypes.⁷⁹

It can be expected that with the development of these techniques and the possibilities of advanced analysis the links between asthma and allergy genes, environmental factors and the development of asthma will be unravelled in the future.

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